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INTERACTION BETWEEN SOME ANTIBIOTICS AND ANTIOXIDANTS

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Abstract

Introduction: The bactericidal action of many antibacterial agents has a common mechanism associated with the generation of hydroxyl radicals and the activation of oxidative stress. This is confirmed by the ratio between the degree of bactericidal effect and the level of hydroxyl radicals. The study of the effect of antioxidants on the antibacterial activity of chemotherapeutic agents can open new prospects for improving the treatment of infectious diseases.

Objectives: Study of the effect of antioxidants on the activity of chemotherapeutic agents in relation to opportunistic bacteria

Methods: The study examined the interaction of antibiotics (gentamicin, ciprofloxacin, ceftazidime) and antioxidants (ascorbic acid, N-acetylcysteine, methylethylpyridinol) in vitro and in vivo. We conducted a dynamic study of the effect of antioxidants at concentrations of 0.5, 1, 2 and 4 mM on the activity of antibacterial agents in vitro for three strains of Klebsiella pneumoniae and three strains of Escherichia coli. The effect of antioxidants on the effectiveness of antibacterial therapy was studied in Wistar rats with experimental bacterial peritonitis.

Results and Discussion: In vitro studies have shown that all antioxidants reduce the activity of gentamicin. Methyl ethylpyridinol increases the effect of ciprofloxacin, ceftazidime. Ascorbic acid enhances the action of ceftazidime. Ascorbic acid, methylethylpyridinol and N-acetylcysteine (80 mg / kg) reduce the antibacterial activity of gentamicin (30 mg / kg) and ciprofloxacin (50 mg / kg) and do not change the intensity of peroxidation processes in combination with these antibacterial agents under experimental infection caused by K. pneumoniae. These antioxidants reduce the prooxidant effect of ciprofloxacin (50 mg / kg), without affecting its antibacterial activity in escherichiosis peritonitis. Ascorbic acid, methylethylpyridinol and N-acetylcysteine (80 mg / kg) do not reduce the nephrotoxic effect of gentamicin (30 mg / kg).

Conclusion: Antioxidants have anmultidirectional effect on the efficacy of antibacterial agents in vitro and under experimental bacterial peritonitis. Combination of antibiotics with antibacterial agents should be accompanied by preliminary in vitro studies. The rational combination of antibacterial agents and antioxidants increases the effectiveness of anti-infective chemotherapy and prevents the formation of resistant strains.

Keywords: antibacterial agents, antioxidants, Klebsiella pneumoniae, Escherichia coli, drug interactions.

Introduction

The use of antibacterial agents has caused the emergence and wide spread of resistant microorganisms, in some cases resistant to several classes of antibacterial drugs [1, 2, 3]. Many researchers note that if existing trends do

not change in a positive way, then medicine will face a problem half a century old, when antibiotics were not yet available [4, 5]. In this regard, the improvement of antimicrobial chemotherapy, aimed at increasing the effectiveness of antibacterial drugs and reducing the number of strains with multiple drug resistance, remains a very urgent task. Pharmacotherapy of a bacterial infection is usually not limited to the use of an antibacterial agent and includes other drugs whose effects are directed to the macroorganism. Antioxidants are used in the pharmacotherapy of bacterial infections because of the significant role of enhancing the processes of free radical oxidation in pathogenesis [6, 7, 8]. The use of the latter is considered justified, since bacterial infection is accompanied by the production of active forms of oxygen damaging biomolecules and making a significant contribution to the development of disorders of cellular metabolism [9, 10, 11]. According to modern data, the bactericidal action of many antibacterial agents has a common mechanism associated with the generation of hydroxyl radicals and the activation of oxidative stress in bacterial cells. This is confirmed by the ratio between the degree of bactericidal effect and the level of hydroxyl radicals [12, 13]. In addition, radical particles in low concentrations act as intracellular signaling molecules, supporting the normal course of biochemical processes [14, 15, 16]. Consequently, antioxidants can reduce the antimicrobial activity of chemotherapeutic agents and reduce the effectiveness of treatment [17]. Thus, the advisability of using antioxidants in combination with antibiotics is controversial. The study of the effect of antioxidants on the antibacterial activity of chemotherapeutic agents is relevant and can open new prospects for improving the treatment of infectious patients.

Objectives

Study of the influence of antioxidants on the activity of chemotherapeutic agents with respect to opportunistic bacteria (Escherichia coli and Klebsiella pneumoniae).

Methods

We studied the interaction of antioxidants and antibiotics (Table 1). The invitro studies used periodic cultures of control and clinical strains of the Enterobacteriaceae family: Escherichiacoli (control strain ATCC 25922 and two clinical strains) and Klebsiella pneumoniae (control strain ATCC 13883 and two clinical strains). Daily cultures of the strains were prepared by incubation on slanted agar at 35 ° C and used to prepare inoculum – bacterial suspensions in a 0.9% sodium chloride solution with an optical density of 1.0 McFarland.

Table 1

Antioxidants and antibiotics

Antioxidants	Antibiotics
Ascorbic acid (Panreac, Spain)	Gentamicin (AppliChem, Germany)
N-acetylcysteine (Sigma-Aldrich, USA)	Ciprofloxacin (Sigma-Aldrich, USA)
Methylethylpyridinol (MEZ, Russia)	Ceftazidime (Sigma-Aldrich, USA)

Incubation mixtures for studying the effect of antioxidants on the activity of antibiotics were prepared on the basis of the mineral medium M9. The incubation mixture contained an antioxidant, an antibiotic and a periodic bacterial culture. Antioxidants were studied at concentrations of 0.25, 0.5, 1, 2 and 4 mM. Antibiotics were kept at concentrations equal to half the minimum inhibitory concentrations established for each strain. After inoculating the bacterial suspension, the mixture containing the antioxidants was incubated in an air thermostat at 35 ° C. for 24 hours. To assess the growth of strains, a device was used to determine the optical density of bacterial suspensions of Densi-la-meter (Erba Lachema s.r.o., Czech Republic). The measurements were carried out every 2 hours for 12 hours, and also after 24 hours of incubation. The obtained data were compared with the data of control incubation mixtures containing no antioxidants.

In vivo studies were performed on 595 male Wistar rats aged 2-3 months weighing

190-290 g grown in the nursery of Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia). The maintenance of animals met the requirements of the Helsinki Declaration of the World Medical Association (2000), the European Convention on the Protection of Vertebrates used for experimental or other scientific purposes (Strasbourg, 1986).

All animals were kept in the same room in individual cages at an air temperature of 20-25 ° C without restriction of access to food and water. To carry out the research, healthy animals were selected who had undergone a two-week quarantine in the vivarium. The experimental groups were formed by random sampling, taking into account the body weight as the determining index.

The in vivo experiments were divided into 2 parts according to the number of microorganisms studied (Escherichia coli, Klebsiella pneumoniae). Each part was divided into 5 series in accordance with the antibacterial agents studied. For each series of 35 animals, groups (1-5) of 7 individuals were formed.

In each series, the animals of all groups were injected intraperitoneally with a bacterial suspension (2.5 units by McFarland, 5 ml / kg) obtained from a daily pure culture of a clinical strain of Escherichia coli or Klebsiella pneumoniae. 3 hours after injection, a sterile solvent of antibacterial agent was administered to animals of Group 1, Group 2 – solution of antibacterial agent, Group 3 – sequential solutions of antibacterial agent and ascorbic acid, Group 4 – solutions of antibacterial agent and methylethylpyridinol, Group 5 – solutions of antibacterial agent and N-acetylcysteine. Antibiotics were administered in doses: gentamicin 30 mg/kg, ciprofloxacin 50 mg/kg, ceftazidime 120 mg/kg. Antioxidants were administered in a dose 80 mg/kg. After 18 hours, the blood of experimental animals underwent biochemical studies.

To assess the free radical oxidation in the blood plasma of rats, the concentrations of ceruloplasmin, thiobarbiturate-reactive products of the plasma were measured, in erythrocytes – the concentration of reduced glutathione, the activity of glutathione peroxidase and catalase. Ceruloplasmin was also determined as a marker of the intensity of the inflammatory process.

To assess liver function in blood plasma, the activity of alanine aminotransferase and alkaline phosphatase was determined. The renal function was assessed by plasma concentrations of urea and creatinine. These parameters were determined using diagnostic kits (Vital Diagnostics SPb, Russia).

Computer programs Microsoft Office Excel 2003 (Microsoft Corporation, USA) and SigmaStat 3.5 (Systat Software Inc., USA) for Windows were used for calculations. The statistical processing of the results was carried out using the nonparametric criteria of Kraskel-Wallis and Mann-Whitney. The data were presented as a median and interquartile range – Me (25%, 75%). Differences were considered significant at $P \leq 0.05$ (comparison of two independent groups) and at $P \leq 0.017$ (pairwise comparison of three independent groups).

Results and Discussion

Interaction between antibacterial agents and antioxidants in vitro

The results of a study of the modulation of the activity of antibacterial agents by antioxidants in vitro are presented in Table 2.

There is a pronounced antagonism between gentamicin and antioxidants, which indicates an important role of enhancing the processes of peroxidation in the course of the bactericidal action of the antibiotic. Note that aminoglycosides – this is the only class of inhibitors of protein biosynthesis, which has a bactericidal effect. This is confirmed by M.A. Kohansky et al. (2007), which proved the leading role of free radical oxidation in the realization of this effect [12].

Glutathione, as a natural metabolite of the microorganisms under consideration, provides universal protection against active forms of oxygen [18]. In connection with this, the expected decrease is the activity of ciprofloxacin and ceftazidime, antibiotics with a proven prooxidant component in the mechanism of action [12], under the action of N-acetylcysteine, a precursor of glutathione.

Table 2

Interaction between antibacterial agents and antioxidants in vitro

Antioxidant	*Escherichiacoli*			*Klebsiellapneumoniae*		
	Strain 1	Strain 2	Strain 3	Strain 1	Strain 2	Strain 3
			Gentamicin			
Ascorbic acid	A++	A++	A++	A++	A++	A++
Methylethylpyridinol	A++	A++	A++	A++	A++	A++
N-acetylcysteine	A++	A++	A++	A++	A++	A++
			Ciprofloxacin			
Ascorbic acid	0	0	0	0	A+	A++
Methylethylpyridinol	S	0	S	S++	S	S
N-acetylcysteine	A++	A++	A++	A	A++	A++
			Ceftazidime			
Ascorbic acid	S	S	S	S	S	S
Methylethylpyridinol	S	S	S	S	S	S
N-acetylcysteine	A++	A++	A++	A++	A++	A+

Note. Antagonism: A ++ – within 24 hours of incubation; A + – in most cases, the dependence on the concentration of the antioxidant may be absent; A – episodic development of culture is higher than control, dependence on antioxidant concentration may be absent; 0 – no influence, the development of culture does not change, or there are point changes with respect to control. Synergy: S – episodic development of culture below control, dependence on antioxidant concentration may be absent; S + – in most cases, the dependence on the concentration of the antioxidant may be absent; S ++ – within 24 hours of incubation.

The effect of ascorbic acid on the activity of antibacterial agents has no obvious picture. The reason for this is the chemical instability of the ascorbic acid molecule under aerobic conditions and the ability of the substance to act as a pro-oxidant [19].

The independent antibacterial activity of methyl ethylpyridinol was demonstrated by us earlier [20]. It has been established that methyl ethylpyridinol enhances the action of all antibacterial agents studied, with the exception of gentamicin, against most strains, with the strongest effect being manifested in high concentrations of the antioxidant. This fact testifies, first, the lack of competition for a pharmacological target, and secondly, the insignificant effect of the antioxidant properties of methyl ethyl pyridinol on the action of pro-oxidants. At the same time, with the incubation with gentamicin, methyl ethyl pyridinol showed the most pronounced antagonistic activity compared to other antioxidants. In this regard, it appears that methylethylpyridinol has an antibacterial mechanism of action similar to aminoglycosides, although less pronounced.

Interaction between antibacterial agents and antioxidants in vivo

Consider the results of a study modulating chemotherapy with the use of **gentamicin** (Tables 3 and 4).It should be noted that this particular antibiotic causes the greatest alertness when administered with antioxidants, since the latter resulted in the appearance of almost complete antibiotic resistance in vitro. The described effect eventually manifested itself in animals with experimental peritonitis – a significant decrease in the concentration of ceruloplasmin is observed only in the regime of monotherapy with an antibiotic, the introduction of antioxidants completely neutralizes this effect. The severity of leaking peritonitis is enhanced by renal dysfunction developing in animals receiving gentamicin due to the implementation of the nephrotoxic properties of the antibiotic.

Table 3

Biochemical indices of rats with bacterial peritonitis caused by Escherichia coli (with gentamicin administration)

P	Group 1	Group 2	Group 3	Group 4	Group 5
			Erythrocyte catalase activity, %/mg Hb		
	0.030 (0.026;0.043)	0.049 (0.045;0.054)	0.041 (0.037;0.047)	0.036 (0.029;0.060)	0.046 (0.043;0.060)
P	x	***0.038***	*0.097*	*0.383*	***0.038***
			Concentration of reduced glutathione in erythrocytes,umol/g Hb		
	0.56 (0.49;0.61)	0.78 (0.68;0.79)	0.74 (0.56;0.82)	0.70 (0.63;0.77)	0.70 (0.55;0.73)
P	x	***0.004***	*0.097*	***0.026***	*0.128*
			Erythrocyte glutathione peroxidase activity,umol/(min× g Hb)		
	18.11 (17.37;20.80)	19.62 (17.61;22.31)	17.15 (13.48;21.54)	18.84 (16.72;20.80)	19.32 (17.28;22.36)
P	x	*0.710*	*0.535*	*1.000*	*0.620*
			Concentration of thiobarbiturate-reactive products,umol/l		
	4.88 (3.26;5.11)	4.65 (3.63;5.88)	4.26 (3.73;4.61)	3.76 (3.44;4.41)	3.64 (3.22;4.24)
P	x	*0.710*	*0.805*	*0.535*	*0.535*
			Concentration of ceruloplasmin,mg/l		
	512.8 (481.7;571.8)	455.0 (418.0;489.1)	511.0 (440.1;523.5)	518.0 (499.2;598.5)	522.4 (414.5;541.8)
P	x	***0.017***	*0.456*	*0.535*	*0.902*
			Alanine aminotransferase activity in plasma,umol/(sec×l)		
	1.13 (0.85;1.48)	1.22 (0.77;1.29)	1.21 (1.02;1.36)	1.26 (1.08;1.59)	1.28 (0.96;1.40)
P	x	*0.902*	*1.000*	*0.620*	*1.000*
			Activity of alkaline phosphatase in plasma,nmol/(sec×l)		
	1758 (1609;1920)	1517 (1287;1713)	1665 (1124;1744)	1625 (1238;1903)	1517 (1378;1638)
P	x	*0.073*	*0.209*	*0.383*	*0.073*
			Urea concentration in plasma,mmol/l		
	2.48 (2.23;2.67)	5.08 (4.14;7.03)	4.26 (3.62;4.65)	5.13 (4.06;6.47)	4.89 (3.74;5.21)
P	x	***<0.001***	***<0.001***	***<0.001***	***0.011***
			Concentration of creatinine in plasma,umol/l		
	66.3 (60.7;70.3)	76.4 (75.3;94.5)	80.4 (72.9;88.3)	89.9 (77.8;110.0)	93.9 (81.9;106.2)
P	x	***0.001***	***0.026***	***0.002***	***0.011***

The administration of gentamicin, as well as its combination with methylethylpyridinol, prevents the formation of a deficit of reduced glutathione in animals with E. coli-induced peritonitis. In animals with peritonitis caused by Klebsiella pneumoniae, this effect is absent. However, neither the antibiotic nor its combination with antioxidants in both cases reduces the concentration of thiobarbiturate-reactive products in plasma under experimental peritonitis. This can be due, firstly, to the pro-oxidant properties of gentamicin, which do not allow to reduce the intensity of the processes of free radical oxidation even when the pathogen is destroyed, and secondly, the renal function is impaired, and accordingly, the elimination of lipid peroxidation products.

Table 4

Biochemical indices of rats with bacterial peritonitis caused by Klebsiella pneumoniae (with gentamicin administration)

P	Group 1	Group 2	Group 3	Group 4	Group 5
		Erythrocyte catalase activity, %/mg Hb			
	0.048 (0.044;0.052)	0.030 (0.023;0.035)	0.049 (0.047;0.052)	0.049 (0.045;0.051)	0.050 (0.044;0.059)
P	x	***0.001***	*0.535*	*0.805*	*0.805*
		Concentration of reduced glutathione in erythrocytes, umol/g Hb			
	0.55 (0.47;0.67)	0.61 (0.45;0.63)	0.46 (0.42;0.57)	0.52 (0.48;0.56)	0.47 (0.45;0.54)
P	x	*1.000*	*0.456*	*0.710*	*0.383*
		Erythrocyte glutathione peroxidase activity, umol/(min× g Hb)			
	18.28 (14.44;19.22)	16.27 (14.01;17.64)	16.92 (16.78;17.48)	17.40 (16.21;18.35)	17.43 (16.47;19.17)
P	x	*0.259*	*1.000*	*0.902*	*0.710*
		Concentration of thiobarbiturate-reactive products, umol/l			
	3.57 (3.43;3.67)	3.49 (2.70;3.59)	3.52 (3.14;3.76)	3.60 (2.96;4.15)	3.45 (3.36;3.92)
P	x	*0.318*	*1.000*	*0.805*	*0.620*
		Concentration of ceruloplasmin, mg/l			
	496.1 (459.6;592.2)	406.0 (382.8;423.7)	462.9 (408.4;528.7)	450.6 (446.0;518.4)	447.1 (438.1;500.5)
P	x	***0.017***	*0.259*	*0.259*	*0.209*
		Alanine aminotransferase activity in plasma, umol/(sec×l)			
	2.32 (1.98;2.56)	1.73 (1.71;1.96)	1.96 (1.76;2.17)	2.27 (1.90;2.31)	1.97 (1.95;2.22)
P	x	***0.017***	*0.128*	*0.383*	*0.318*
		Activity of alkaline phosphatase in plasma, nmol/(sec×l)			
	1493 (1281;1558)	1301 (1183;1486)	1286 (1210;1492)	1304 (1221;1482)	1431 (1222;1522)
P	x	*0.318*	*0.383*	*0.383*	*0.456*
		Urea concentration in plasma, mmol/l			
	2.25 (1.94;2.56)	4.67 (4.48;5.15)	4.81 (4.60;5.39)	5.29 (4.40;5.62)	4.81 (4.41;5.40)
P	x	***<0.001***	***<0.001***	***<0.001***	***<0.001***
		Concentration of creatinine in plasma, umol/l			
	55.8 (49.4;60.0)	95.6 (88.3;105.7)	96.1 (89.5;102.4)	98.1 (90.9;107.6)	96.9 (86.1;105.0)
P	x	***<0.001***	***<0.001***	***<0.001***	***<0.001***

The administration of **ciprofloxacin** reduces the oxidase activity of ceruloplasmin, and this effect in rats with peritonitis induced by E. coli, is preserved both in the monochemotherapy regimen and in co-administration with antioxidants (Table 5).

Table 5

Biochemical indices of rats with bacterial peritonitis caused by Escherichia coli (with ciprofloxacin administration)

P	Group 1	Group 2	Group 3	Group 4	Group 5
			Erythrocyte catalase activity, %/mg Hb		
	0.042 (0.033;0.051)	0.038 (0.035;0.044)	0.039 (0.028;0.048)	0.042 (0.030;0.050)	0.038 (0.029;0.055)
P	x	*0.456*	*0.318*	*0.535*	*0.710*
			Concentration of reduced glutathione in erythrocytes, umol/g Hb		
	0.55 (0.52;0.65)	0.73 (0.65;0.76)	0.72 (0.63;0.82)	0.63 (0.50;0.88)	0.62 (0.54;0.67)
P	x	***0.011***	***0.026***	*0.456*	*0.456*
			Erythrocyte glutathione peroxidase activity, umol/(min× g Hb)		
	13.31 (12.92;16.99)	14.69 (12.26;17.07)	18.90 (16.07;19.74)	19.39 (17.55;20.41)	19.23 (17.78;21.12)
P	x	*0.902*	***0.026***	***0.038***	***0.026***
			Concentration of thiobarbiturate-reactive products, umol/l		
	4.69 (4.52;4.92)	3.95 (3.59;4.49)	4.65 (4.53;4.68)	3.64 (3.39;4.42)	4.11 (3.75;4.31)
P	x	***0.017***	*0.535*	***0.004***	***0.004***
			Concentration of ceruloplasmin, mg/l		
	536.4 (499.8;601.3)	383.3 (350.4;465.7)	471.6 (393.1;491.8)	461.1 (437.5;476.7)	410.4 (394.4;488.9)
P	x	***0.004***	***0.026***	***0.017***	***0.026***
			Alanine aminotransferase activity in plasma, umol/(sec×l)		
	1.93 (1.84;2.53)	1.87 (1.83;1.90)	1.77 (1.62;2.09)	1.80 (1.74;1.99)	1.77 (1.67;1.96)
P	x	*0.383*	*0.209*	*0.165*	*0.128*
			Activity of alkaline phosphatase in plasma, nmol/(sec×l)		
	1458 (1438;1866)	1464 (1397;1530)	1304 (1280;1589)	1458 (1292;1560)	1319 (1250;1821)
P	x	*0.456*	*0.259*	*0.456*	*0.165*
			Urea concentration in plasma, mmol/l		
	1.56 (1.41;2.08)	1.78 (1.55;2.08)	1.93 (1.72;2.03)	1.78 (1.64;1.89)	1.62 (1.57;1.80)
P	x	*0.456*	*0.383*	*0.383*	*0.805*
			Concentration of creatinine in plasma, umol/l		
	46.8 (45.2;49.3)	51.8 (48.9;54.5)	50.8 (49.7;53.1)	51.3 (48.6;53.0)	50.3 (50.1;53.2)
P	x	*0.165*	*0.209*	*0.259*	*0.165*

The concentration of thiobarbiturate-reactive products in animals with E. coli-induced peritonitis decreases with ciprofloxacin, indicating a decrease in the intensity of peroxidation processes. This effect is observed despite the known pro-oxidant properties of ciprofloxacin. In this regard, it can be argued that a decrease in the intensity of peroxidation is associated with the antibacterial action of ciprofloxacin, and the elimination of products of peroxidation and endotoxins is not hampered by the preserved functions of the liver and kidneys. Important is the fact that the administration of antioxidants increases the activity of glutathione peroxidase. Untypical is the profile of peroxidation markers in animals with escherichial peritonitis who received ciprofloxacin with ascorbic acid. On the one

hand, the concentration of thiobarbiturate-reactive products is maintained at the control level, which indicates the lack of realization of its antioxidant properties by ascorbic acid. On the other hand, the concentration of reduced glutathione is at the same level as the monotherapy group. This is probably due to the dual properties of ascorbic acid, which, depending on the conditions, can be both anti- and pro-oxidant.

The administration of antioxidants to animals with K. pneumoniae infection retains the activity of ceruloplasmin at the control level, which indirectly may indicate a decrease in the effectiveness of treatment (Table 6).

Table 6

Biochemical indices of rats with bacterial peritonitis caused by Klebsiella pneumonia (with ciprofloxacinadministration)

P	Group 1	Group 2	Group 3	Group 4	Group 5
			Erythrocyte catalase activity, %/mg Hb		
	0.053	0.046	0.046	0.040	0.048
	(0.048;0.057)	(0.036;0.049)	(0.034;0.048)	(0.038;0.050)	(0.039;0.053)
P	x	***0.038***	***0.026***	***0.038***	*0.209*
			Concentration of reduced glutathione in erythrocytes, umol/g Hb		
	0.64	0.63	0.68	0.84	0.66
	(0.61;0.72)	(0.59;0.69)	(0.63;0.80)	(0.59;0.87)	(0.63;0.81)
P	x	*0.620*	*0.318*	*0.383*	*0.535*
			Erythrocyte glutathione peroxidase activity, umol/(min× g Hb)		
	11.63	16.94	13.04	12.35	15.11
	(10.37;15.27)	(14.26;18.44)	(11.89;14.10)	(11.06;14.03)	(13.16;15.25)
P	x	*0.097*	*0.535*	*0.805*	*0.620*
			Concentration of thiobarbiturate-reactive products, umol/l		
	4.26	4.26	4.34	4.34	3.84
	(3.71;4.93)	(3.74;4.84)	(3.91;4.47)	(3.94;4.47)	(3.71;4.36)
P	x	*0.805*	*0.902*	*0.902*	*0.535*
			Concentration of ceruloplasmin, mg/l		
	468.1	427.0	406.0	415.6	445.4
	(446.3;529.8)	(399.9;446.3)	(404.0;439.5)	(397.0;491.3)	(424.6;468.3)
P	x	***0.038***	*0.053*	*0.165*	*0.209*
			Alanine aminotransferase activity in plasma, umol/(sec×l)		
	1.99	1.86	2.25	2.08	2.03
	(1.94;2.40)	(1.73;2.00)	(2.00;2.56)	(1.83;2.40)	(1.74;2.25)
P	x	*0.165*	*0.535*	*1.000*	*0.456*
			Activity of alkaline phosphatase in plasma, nmol/(sec×l)		
	1515	1328	1541.8	1325	1524
	(1368;1942)	(1287;1619)	(1361;1718)	(1270;1757)	(1316;1641)
P	x	*0.209*	*0.620*	*0.318*	*0.620*
			Urea concentration in plasma, mmol/l		
	1.98	1.96	1.99	2.23	2.01
	(1.88;2.30)	(1.89;2.29)	(1.86;2.11)	(1.76;2.35)	(1.87;2.29)
P	x	*0.902*	*0.805*	*0.902*	*0.902*
			Concentration of creatinine in plasma, umol/l		
	48.3	45.1	55.3	57.1	53.8
	(46.6;55.5)	(45.1;50.2)	(45.6;56.2)	(46.1;57.5)	(47.7;59.1)
P	x	*0.128*	*1.000*	*0.710*	*0.620*

Table 7

Biochemical indices of rats with bacterial peritonitis caused by Escherichia coli (with ceftazidimeadministration)

P	Group 1	Group 2	Group 3	Group 4	Group 5
		Erythrocyte catalase activity, %/mg Hb			
	0.048 (0.035;0.051)	0.027 (0.019;0.038)	0.029 (0.026;0.032)	0.031 (0.021;0.033)	0.031 (0.029;0.035)
P	x	***0.026***	***0.001***	***0.007***	***0.017***
		Concentration of reduced glutathione in erythrocytes, umol/g Hb			
	0.52 (0.38;0.58)	0.67 (0.56;0.76)	0.67 (0.62;0.74)	0.68 (0.64;0.72)	0.68 (0.67;0.71)
P	x	***0.038***	***0.038***	***0.017***	***0.011***
		Erythrocyte glutathione peroxidase activity, umol/(min× g Hb)			
	16.77 (14.48;17.72)	19.07 (18.11;22.71)	20.09 (19.73;22.26)	21.57 (19.71;22.11)	21.48 (18.63;22.91)
P	x	***0.007***	***0.002***	***0.002***	***0.026***
		Concentration of thiobarbiturate-reactive products, umol/l			
	4.38 (3.89;4.60)	2.79 (2.34;3.71)	2.87 (2.51;3.30)	3.29 (3.23;3.58)	2.91 (2.27;3.49)
P	x	***0.038***	***0.011***	***0.038***	***0.011***
		Concentration of ceruloplasmin, mg/l			
	497.9 (462.9;509.5)	389.4 (331.2;414.3)	443.6 (328.3;529.6)	357.0 (314.1;456.1)	347.4 (291.8;400.5)
P	x	***0.038***	*0.456*	*0.128*	***0.007***
		Alanine aminotransferase activity in plasma, umol/(sec×l)			
	1.32 (1.27;1.64)	1.23 (1.17;1.29)	1.10 (0.85;1.25)	1.11 (0.96;1.22)	1.11 (0.87;1.18)
P	x	***0.026***	***0.007***	***0.004***	***<0.001***
		Activity of alkaline phosphatase in plasma, nmol/(sec×l)			
	1193 (1103;1722)	1301 (1179;1763)	1301 (1153;1532)	1295 (1216;1370)	1310 (1207;1383)
P	x	*0.710*	*0.902*	*0.710*	*0.805*
		Urea concentration in plasma, mmol/l			
	1.84 (1.74;1.91)	1.77 (1.76;1.86)	1.73 (1.71;1.97)	1.66 (1.65;1.71)	1.72 (1.63;2.30)
P	x	*1.000*	*0.535*	*0.128*	*0.620*
		Concentration of creatinine in plasma, umol/l			
	54.8 (44.2;54.8)	50.6 (42.1;58.0)	50.6 (40.1;54.8)	46.3 (42.1;57.0)	48.3 (42.9;51.8)
P	x	*0.805*	*0.620*	*0.620*	*0.318*

The administration of ciprofloxacin, as well as its combination with antioxidants, does not lead to a decrease in the concentration of thiobarbiturate-reactive products and an increase in the content of reduced glutathione. The activity and glutathione peroxidase does not change. This indicates a persistent oxidative stress even against antibiotic chemotherapy. The only effect of ciprofloxacin (including in combination with ascorbic acid and methylethylpyridinol) is a decrease in catalase activity. Thus, despite a decrease in the plasma concentration of ceruloplasmin, ciprofloxacin does not cause a decrease in oxidative stress in animals with peritonitis caused by Klebsiella pneumoniae. Probably, this is due to its more severe course compared to infection caused by E. coli. Antioxidants also had a weak effect.

Hence it should be concluded that the effectiveness of the use of antioxidants in the complex therapy of bacterial infection depends not only on the mechanism of action of the antibacterial agent, but also on the specific pathogen.

Just like ciprofloxacin, **ceftazidime** causes a decrease in oxydase activity of ceruloplasmin, but its combination with antioxidants affect this parameter ambiguously (Tables 7 and 8).

In particular, the effect of the antibiotic is preserved in combination with ascorbic acid and methylethylpyridinol, but is lost in combination with N-acetylcysteine. In animals with an escherichiosis infection, on the contrary, the effect of ceftazidime is retained only in combination with N-acetylcysteine.

Table 8

Biochemical indices of rats with bacterial peritonitis caused by Klebsiella pneumoniae (with ceftazidimeadministration)

P	Group 1	Group 2	Group 3	Group 4	Group 5
			Erythrocyte catalase activity, %/mg Hb		
	0.042 (0.035;0.057)	0.018 (0.015;0.024)	0.028 (0.021;0.030)	0.028 (0.021;0.034)	0.033 (0.028;0.036)
P	x	***0.002***	***0.004***	***0.011***	*0.073*
			Concentration of reduced glutathione in erythrocytes, umol/g Hb		
	0.51 (0.45;0.60)	0.68 (0.60;0.74)	0.56 (0.53;0.67)	0.60 (0.53;0.74)	0.60 (0.50;0.66)
P	x	***0.007***	*0.128*	*0.128*	*0.318*
			Erythrocyte glutathione peroxidase activity, umol/(min× g Hb)		
	18.52 (17.44;19.98)	20.95 (19.07;22.38)	20.19 (18.37;21.36)	20.98 (19.12;21.57)	19.02 (18.20;19.42)
P	x	*0.053*	*0.209*	*0.053*	*0.710*
			Concentration of thiobarbiturate-reactive products, umol/l		
	3.80 (3.50;4.58)	3.10 (2.84;3.53)	3.33 (3.27;3.72)	3.53 (3.22;3.78)	3.33 (3.27;3.60)
P	x	***0.017***	*0.097*	*0.165*	*0.073*
			Concentration of ceruloplasmin, mg/l		
	500.5 (425.9;532.0)	406.0 (381.3;416.9)	383.3 (356.6;409.7)	380.6 (367.3;400.3)	409.5 (388.3;432.3)
P	x	***0.026***	***0.011***	***0.011***	*0.073*
			Alanine aminotransferase activity in plasma, umol/(sec×l)		
	1.89 (1.77;2.25)	1.52 (1.25;1.76)	1.69 (1.30;1.82)	1.73 (1.27;1.75)	1.76 (1.21;1.81)
P	x	***0.026***	*0.073*	*0.053*	***0.038***
			Activity of alkaline phosphatase in plasma, nmol/(sec×l)		
	1557 (1315;1748)	1389 (1187;1442)	1404 (1302;1493)	1572 (1306;1751)	1464 (1292;1628)
P	x	*0.209*	*0.318*	*0.902*	*0.456*
			Urea concentration in plasma, mmol/l		
	1.81 (1.60;2.33)	2.04 (1.96;2.12)	2.27 (1.93;2.54)	2.17 (1.91;2.36)	2.14 (2.08;2.39)
P	x	*1.000*	*0.209*	*0.383*	*0.259*
			Concentration of creatinine in plasma, umol/l		
	45.3 (43.7;47.9)	44.81 (44.2;46.0)	47.6 (46.6;49.9)	47.8 (46.4;51.1)	51.3 (48.6;52.7)
P	x	*0.902*	*0.097*	*0.097*	*0.073*

A characteristic effect that is caused by ceftazidime is a decrease in plasma activity of alanine aminotransferase, which indicates a decrease in liver damage in conditions of peritonitis, and is probably also a consequence of the antioxidant effect of the antibiotic.

To confirm this, consider the peroxidation markers. In animals under the influence of ceftazidime, the concentration of thiobarbiturate-reactive products actually decreases. In this case, in combination with antioxidants, the effect does not increase in animals with escherichial peritonitis, and in animals with peritonitis caused by Klebsiella pneumoniae, on the contrary, it is lost. Similar changes are observed when considering the concentration of reduced glutathione.

In animals with peritonitis caused by Klebsiella pneumoniae, antibiotic and its combination with antioxidants cause a decrease in the activity of erythrocyte catalase and an increase in the activity of glutathione peroxidase. With infection caused by Klebsiella pneumoniae, the activity of the latter does not change, and the combination with N-acetylcysteine retains catalase activity at the control level.

Thus, antioxidants in complex therapy of bacterial infection using ceftazidime have an ambiguous effect, which is due to the properties of the pathogen strain.If they did not affect the activity of the antibiotic in the therapy of escherichial peritonitis, then in the case of infection caused by Klebsiella pneumoniae, there is no definite regular influence. In addition, because of its instability, ceftazidime can have a very unstable pharmacokinetics. However, the decrease in antibiotic activity caused only N-acetylcysteine and only under conditions of infection caused by Klebsiella pneumoniae. This confirms the data obtained by us in vitro and demonstrates a decrease in the bactericidal effect of ceftazidime by N-acetylcysteine.

Conclusion

In vitro studies found that all antioxidants significantly reduce the activity of gentamicin. N-acetylcysteine also reduces the activity of ciprofloxacin and ceftazidime. Methylethylpyridinol increases the effect of ciprofloxacin, ceftazidime. Ascorbic acid enhances the action of ceftazidime.

Ascorbic acid, methylethylpyridinol and N-acetylcysteine (80 mg/kg) reduce the antibacterial activity of gentamicin (30 mg/kg) and ciprofloxacin (50 mg/kg) and do not change the intensity of peroxidation processes when combined with these antibacterial agents under experimental infection conditions, caused by K. pneumoniae. These antioxidants reduce the pro-oxidant effect of ciprofloxacin (50 mg / kg) without affecting their antibacterial activity under experimental escherichiosis infection. Ascorbic acid, methylethylpyridinol and N-acetylcysteine

(80 mg/kg) do not reduce the nephrotoxic effect of gentamicin (30 mg/kg), but decrease its antibacterial efficacy.

The use of substances that alter the activity of antibacterial agents has not only an obvious clinical, but also epidemiological significance. The use of any antibacterial agent leads to a gradual increase in the frequency of resistance of microorganisms. The time interval from the beginning of application of the drug to the moment of occurrence of 100% resistance is the time of effective use of the antibacterial agent. Inclusion in the chemotherapy of modulators-synergists, increasing the effectiveness of treatment, leads to an extension of this time. Modulators-antagonists, which reduce the activity of antibacterial agent, reduce the time of its effective use.

The activity of each antioxidant essentially depends on the environment and the conditions of its functioning [21]. Another reason complicating the evaluation of antioxidant defense mechanisms is the interdependence of the content and activity of antioxidants combined into antioxidant systems. The effect of some antioxidants can be inverted when their content changes. In this regard, the effects exhibited by antioxidants are determined by the applied dose, by administration, biotransformation and elimination.

The obtained data demonstrate that the existing traditional approach to determining the expediency of prescribing an antibacterial agent by microbiological evaluation of the susceptibility to the pathogen is insufficient in the context of complex pharmacotherapy. This

is due, above all, to the fact that other drugs can significantly change the sensitivity of bacteria to the chemotherapeutic agent until the development of resistance. Therefore, in order to increase the effectiveness of pharmacotherapy and reduce the occurrence of antibiotic resistant strains, it is necessary to conduct in vitro microbiological studies with all drugs planned for the treatment of the patient, as well as the causative agent that caused the disease, since the type of interaction of antibacterial agents and modulator substances is determined not only by mechanisms the effects of these drugs, but also the characteristics of a particular strain of the microorganism.

Conflicts of Interest

The authors have no conflict of interest to declare.

References

1. Hoban DJ, Nicolle LE, Hawser S, Bouchillon S, Badal R.Antimicrobial susceptibility of global inpatient urinary tract isolates of Escherichia coli: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program: 2009-2010. *Diagn Microbiol Infect Dis.* 2011;70(4):507-511. [PubMed] [Full Text]

2. Rice, L. B. The clinical consequences of antimicrobial resistance.*Curr. Opin. Microbiol*. 2009;12:476-481. [PubMed] [Full Text]

3. Kozlov RS, Golub AV, Dekhnich AV, Sukhorukova MV.Antimicrobial Resistance of Gram-negative Microorganisms Causing Complicated Intra-abdominal Infections in Russia. *Clinical microbiology and antimicrobial chemotherapy. [Klinicheskaya mikrobiologiya i antimikrobnaya khimioterapiya]*. 2015;3:227-234. (in Russian) [eLIBRARY] [Full Text]

4. Kosinets AN, Frolova AV, Bulavkin VP, Okulich VK.Antibiotic resistance. New opportunities for antibacterial action.*Bulletin of Vitebsk State Medical University.[Vestnik Vitebskogo gosudarstvennogo meditsinskogo universiteta]*. 2014;13(2):70-77. (in Russian) [eLIBRARY] [Full Text]

5. Kapil A. The chalenge of antibiotic resistance: need to contemplate.*Indian J. Med. Res.*2005;121(2):83-91. [PubMed]

6. Nechayeva GI, Vershinina MV, Vysokogorskiy VE, Pritykina TV.Substantiation of the need for antioxidant therapy in community-acquired pneumonia in patients with connective tissue dysplasia.*Proceedings of the Chelyabinsk Scientific Center of the URO RAS. [Izvestiya Chelyabinskogo nauchnogo tsentra URO RAN]*. 2004;1(22):220-224. (In Russian) [eLIBRARY] [Full Text]

7. Nevskaya NA.Antioxidants in intensive therapy of peritonitis. *Far Eastern Medical Journal*. *[Dal'nevostochnyy meditsinskiy zhurnal]*. 2009;4:126-130. (In Russian) [eLIBRARY] [Full Text]

8. Lowes DA, Webster NR, Murphy MP, Galley HF.Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress, improve mitochondrial function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. *British Journal of Anaesthesia*. 2013;110(3):472-480. [PubMed] [Full Text]

9. Exline MC, Crouser ED.Mitochondrial mechanisms of sepsis induced organ failure.*Frontiers Biosc*. 2008;13:5031-5041. [PubMed] [Full Text]

10. Ding S, Minohara Y, Fan XJ [et al.].Helicobacter pylori infection induces oxidative stress and programmed cell death in human gastric epithelial cells. *Infection and Immunity*. 2007. [PubMed] [Full Text]

11. Ruggieri AJ, Levy RJ, Deutschman CS.Mitochondrial dysfunction and resuscitation in sepsis. *Crit. Care Clin*. 2010;26:567-575. [PubMed] [Full Text]

12. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell*. 2007;130:797-810. [PubMed] [Full Text]

13. Dwyer DJ, Kohanski MA, Hayete B, Collins JJ. Gyrase inhibitors induce an oxidative damage cellular death pathway in Escherichia coli. *Molecular Systems Biology*. 2007;3:91.[PubMed] [Full Text]

14. D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that

generate specificity in ROS homeostasis. Nature Reviews Molecular Cell Biology. 2007;8:813-824. [PubMed] [Full Text]

15. Bledsoe G, Crickman S, Mao J, Xia CF, Murakami H, Chao L, Chao J.Kallikrein/kinin protects against gentamicin-induced nephrotoxicity by inhibition of inflammation and apoptosis. *Nephrology Dialysis Transplantation*. 2006;21:624-633. [PubMed] [Full Text]

16. Tonks NK. Redox redux: revisiting PTPs and the control of cell signaling.*Cell*. 2005;121:667-670. [PubMed] [Full Text]

17. Goswami M, Mangoli SH, Jawali N. Effects of glutathione and ascorbic acid on streptomycin sensitivity of Escherichia coli. *Antimicrobial agents and chemotherapy*. 2007;51(3):1119-1122. [PubMed] [Full Text]

18. Große C, Schleuder G, Schmole C, Nies DH. Survival of Escherichia coli cells on solid copper surfaces is increased by glutathione. *Applied and Environmental Microbiology*. 2014;80(22):7071-7078. [PubMed] [Full Text]

19. Matsuoka Y, Yamato M, Yamada K.Fluorescence probe for the convenient and sensitive detection of ascorbic acid. *J. Clin. Biochem. Nutr.* 2016; 58(1):16-22. [PubMed] [Full Text]

20. Miroshnichenko AG, Bryukhanov VM, Butakova LY, Gossen IY, Perfil'yev VY, Smirnov PV.Antioxidants effect on the growth of the culture of escherichia coli and sensitivity to gentamicin. *Fundamental research. [Fundamental'nye issledovaniya]*. 2013;5:339-343. (In Russian) [eLIBRARY] [Full Text]

21. Men'shchikova EB,Lankin VZ, Zenkov NK, Bondar' IA, Krugovykh NF, Trufakin VA. Oxidative stress. Prooxidants and antioxidants(Moscow: Slovo, 2006), 556. (In Russian) [eLIBRARY]

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